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Antioxidant and Antibacterial Activity of Extracts from Lichen *Xanthoparmelia somloensis*, Native to the Black Hills, South Dakota, USA

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ABSTRACT:
The present study was carried out to evaluate the antioxidant and antibacterial activity of lichen *Xanthoparmelia somloensis*, native to the Black Hills in South Dakota, USA. The antioxidant activity of lichen extracts was assessed using the 1,1-diphenyl-2-picryl-hydrazyl free radical scavenging assay. The lipid peroxidation reaction of acetone and methanol extracts was inhibited 85% and 81%, respectively A free radical scavenging activity of 77% (acetone extract) and 65% (methanol extract) was determined. The antibacterial activity was assayed against four clinical strains using the agar well diffusion method. Except for *Escherichia coli*, both extracts were found inhibitory to *Streptomyces aureus*, *Streptococcus pyogenes*,and *Steptococcus agalactiae* with minimum inhibitory concentration values of 0.7-0.9 mg/ml. It was demonstrated that both the antioxidant and antibacterial activities correlated well with the protein to polysaccharide ratio rather than the polyphenol content of the lichen extracts. To the best of our knowledge, this is the first literature report on antibacterial activity from the lichen *X.somloensis.* The results reported here warrant further investigations to establish the usefulness of *X.somloensis* in biomedical applications such as treatment of respiratory and urinary tract infections.

Key words: *Xanthoparmelia somloensis*, lichen extract, antioxidant activity, antibacterial activity

INTRODUCTION

Pathogenic microbes pose serious threats to human health and are increasing in prevalence in institutional health care settings [1]. The challenge for today's pharmaceutical industry lies in the discovery and development of new pharmacologically active products [2] and new antibiotics that are active against resistant bacteria are constantly required. In addition, natural bioactive compounds are proposed as a therapeutic alternative to conventional antimicrobial treatment, whose effectiveness is often limited by the resistance that the infectious agents have developed against antibiotics [3,4].

Lichens are by definition symbiotic plant-like organisms, usually composed of a fungal partner, the mycobiont, and one or more photosynthetic partners, the phytobiont, often either a green alga or cyanobacterium. Lichens and lichen products have been used in traditional medicines for centuries and still hold considerable interest as alternative treatments in various parts of the world. They produce characteristic secondary metabolites that are unique with respect to those of higher plants [5]. The lichen-derived secondary metabolites are grouped in categories, which are distinct from those produced by higher plants. These include: diterpene, triterpene, dibenbenzofuran, dibenzopyranone, depside, depsidones, anthraquinone, xanthones, usnic and pulvinic acids [6]. In various systems of traditional medicine worldwide, lichen species are described to effectively cure dyspepsia, bleeding piles, bronchitis, scabies, stomach disorders, and many disorders of blood and heart [7-9]. Lichen secondary metabolites exhibit numerous biological activities including antimycobacterial [10], antiviral [11], antioxidant [12], analgesic [13], cytotoxic, antimicrobial, fungicidal, herbicidal, antifeedant, and photosystem inhibitory activity [6].

Lichens are important constituents of many ecosystems such as the U.S. National Parks and Forest [14]. Among them, the Black Hills, a coniferous evergreen forest of ponderosa pine located in the southwestern part of South Dakota (Rapid City region), represents a rich eco-center of unverified and untapped lichen diversity. In this study, we evaluated the antioxidant, lipid peroxidation inhibitory, and antibacterial activities of methanol and acetone extracts from a *Xanthoparmelia somloensis* lichen isolate, sampled in the Black Hills area of South Dakota, USA.

MATERIALS AND METHODS

Lichen

Samples of the lichen species *Xanthoparmelia somloensis* grown on rocks at the Sylvan Lake of Black Hills, South Dakota, USA (latitude 43.8475; longitude 103.5657; altitude 6175 ft) were collected in August 2009. The fresh whole lichen material was shade-dried, and mechanically ground into powder-like material. For extraction, 9 g of the lichen powder were added to 200 ml of methanol or acetone. The mixture was subjected to soxhlet extraction for 48 h. The extracts were filtered through Whatman No 1 filter paper, and the filtrates were vacuum-dried with a rotary evaporator (Thermo Scientific, Cincinnati, OH, USA) [15]. The vacuum-dried solids were re-dissolved in dimethylsulfoxide (DMSO) to a final concentration of 0.9 mg dry matter/ml DMSO and then used for determination of the antioxidant and antibacterial activity.

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Antioxidant activity

The free radical scavenging activity of the lichen extracts (0.9 mg dry matter/ml DMSO) was assayed *in vitro* with 1,1-diphenyl-2-picryl-hydrazyl (DPPH) according to Kekuda et al. [16]. One ml of 0.1 mM solution of DPPH in methanol was added to 3 ml of lichen extract (prepared in DMSO as described above) and allowed to stand at room temperature for 30 min. The absorbance was read at 517 nm against blank samples. A lower absorbance of the reaction mixture indicated a higher free radical scavenging activity which was calculated from the DPPH calibration curve.

Antibacterial activity

The test bacteria *E. coli*, *S. aureus S. pyogenes*, and *S. agalactiae* were obtained from the Clinical Laboratory of Rapid City Regional Medical Center, Rapid City, SD, USA. They were screened for their sensitivity towards the lichen extracts using the agar well diffusion method [17]. According to this method, 24 h-old Luria-Burteni (LB) broth cultures of the test bacteria were spread uniformly on solidified sterile LB agar plates using sterile cotton swab. Wells of 6 mm diameter were aseptically bored in the inoculated plates with the help of a cork-borer. The methanol and acetone extracts were vacuum-dried and re-dissolved in DMSO solvent to a final concentration of 0.9 mg/ml (as described above). Subsequently, a 0.1 ml of DMSO-dissolved extracts were introduced in each well of the LB agar plate. Sterile DMSO (0.1 ml), which was introduced into one of the wells of each agar plate, served as control. The plates were incubated in upright position at 37^0C for 24 h. The diameter of the zone of bacterial inhibition around each well was measured and readings were recorded in mm.

Inhibition of lipid peroxidation

The inhibition of lipid peroxidation was determined according to Liegeois et al. [18]. A 30µl aliquot of 16 mM linoleic acid dispersion was added to UV cuvettes containing 2.81 ml of 0.05M phosphate buffer, pH 7.4, and thermo-equilibrated at 40^0C for 10 min. The oxidation reaction was initiated in the presence of 20 µl aliquots of lichen extract (0.9 mg/ml) at 37^0C by adding 150 µl of 40 mM, 2,2'-azobis (2-amidino-propane) dihydrochloride (AAPH) solution. The rate of peroxidation was monitored by recording the increase in absorbance at 234 nm caused by conjugated diene hydroperoxides and expressed as percentage inhibition of lipid peroxidation.

Table 1. Composition and antioxidant activity of extracts from lichen *X. somloensis*

Profile	Units	Methanol extract[1]	Acetone extract[1]
Extract yield	mg/g dry sample	9.00	0.90
Lipid peroxidation inhibition	%	81±0.2	85±0.2
DPPH[2] free radical scavenge activity	%	65±5	76.6±1
Total polyphenol yield	mg/g dry extract	10.1±0.1	4.1±0.1
Protein yield	mg/g dry extract	121.8±1.9	119±2.6
Polysaccharide yield	mg/g dry extract	57±5.8	25±1.2
Protein to polysaccharide ratio	-	2.1±0.2	4.8±0.1

[1]Mean of triplicate ± SD; [2]1,1-diphenyl-2-picryl-hydrazyl

Table 2. Antimicrobial activity of extracts from lichen *X. somloensis*

Clinical test bacteria	Methanol extract[2]	Acetone extract[2]	MIC[3] (mg/ml)
	[1]Zone of inhibition (mm)		
Escherichia coli	-	-	-
Staphylococcus aureus	17±1.3	20±0.6	0.8
Streptococcus pyogenes (Group A)	28±1.1	30±1.3	0.7
Streptococcus agalactiae (Type B)	21±1.3	27±2.1	0.9

[1]Mean of 5 analysis ± SD; [2]Extract concentration (0.9 mg/ml); [3]Minimum inhibitory concentration

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Minimal inhibitory concentration

Increasing concentrations of each extract (0.1 to 1 mg dry matter/ml DMSO) were added to the LB broth and then inoculated with a loopful of cultures of *S. aureus*, *S. pyogenes* and *S. agalactiae* [19]. The minimal inhibitory concentration (MIC) was determined based on the visual turbidity of the culture-extract mixtures following their incubation at 37°C for 24 h. Samples with no turbidity were considered to have the MIC value of the extract for the respective test bacterium.

Analyses

Due to the intimate polysaccharide-protein association [20], their content in the lichen extracts (prepared in DMSO) was determined using phenol-sulfuric acid hydrolysis [21] and Coomassie blue staining [22]. The total soluble phenolics in the mycobiont of lichen extracts were determined with Folin-Ciocalteu reagent and pyrocatechol as standard as described by Behera et al. [23]. A mixture of 0.1 ml of lichen extract and 1 ml of Folin-Ciocalteu reagent was shaken for 5 min, then 3 ml of Na_2CO_3 (2%) was added, and allowed to stand with intermittent shaking for 1 h. Thereafter, the absorbance was measured at 760 nm and the concentration of total polyphenolic compounds in the lichen extracts was determined as µg pyrocatechol equivalents as obtained from the standard pyrocatechol graph. All experimental results were presented as the mean of triplicate determinations with a standard deviation (SD) calculated using Microsoft excel.

RESULTS AND DISCUSSION

The lichen sample, collected from the Black Hills of South Dakota, was identified as *Xanthoparmelia somloensis* (Fig. 1). The extraction yields, composition, and antioxidant/ antibacterial activities of the methanol and acetone extracts of *X. somloensis* are summarized in Table 1 and 2.

Fig. 1. Lichen *X. somloensis* grown in the Black Hills, South Dakota, USA

The methanol and acetone extracts of lichen (0.9 mg/ml) exhibited strong antioxidant activity of 81% and 85%, respectively, during linoleic acid peroxidation. Literature survey reveals that the methanol extract from the lichen *Usnea ghatensis* showed 87% inhibition of lipid peroxidation [24]. Likewise, methanol extracts from *X. somloensis* were reported to inhibit the same reaction by 94% [25].To the best of our knowledge, our study is the second only literature report on antioxidant activities from *X. somloensis*. The percentage inhibition of lipid peroxidation by the *X. somloensis* extracts appeared to be higher when compared to extracts from lichens such as *Arththelium awasthii*, *Heterodermia podocarpa*, and *Parmotrema tinctorum* [26].

The DPPH scavenging activity of the *X. somloensis* extracts was 65% (methanol extract) and 76% (acetone extract) (Table 1) The radical scavenging activity of *X. somloensis* is most likely due to the presence of active principle components in the extracts. Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS [27,28]. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and gallic acid esters have been thought to cause or prompt negative health effects, and subsequently restrictions have been imposed to limit their use [29,30]. In recent years, much attention has been devoted to natural antioxidants and their potential association with health benefits [31]. Phenolics seem to be the largest group of phytochemicals that appear to account for most of the antioxidant activity in plant extracts [32]. Generally, polyphenols have been suggested as the main active ingredient in extracts with strong free radical scavenging activity [33]. For instance, the high antioxidant activity of *X. somloensis* methanol extracts was correlated to their high phenolic content [25]. Our results however do not support these findings. As evident from Table 1, the methanol extract of *X. somloensis* had a higher phenolic content (10.1 mg/g dry extract) than the acetone extract (4.1 mg/ml).

Although the antioxidant activity of the acetone extract was higher than the methanol extract, its polysaccharide content (25 mg/g dry extract) was significantly lower than the methanol extract (57 mg/g). These findings (Table 1) suggest that the polysaccharide content did not appear as a major factor determining the antioxidative activity. The reason for this could be multi-fold as it has been reported that the functional activity of polysaccharides could be affected by the molecular weight, degree of branching, water solubility, structure, and configuration [34,35].

Although the protein content of both extracts was similar, their protein to polysaccharide ratio differed (Table 1). Overall, the antioxidant activity (DPPH scavenging activity and lipid peroxidation inhibition) was higher in the acetone extract than methanol extract. This suggests that the antioxidant activity may be related to the protein to polysaccharide ratio. The results of Liu et al. [36]

indicated that the free radical scavenging activity of polysaccharides was dependant on the ratio of polysaccharides to protein. More respecifically, the ratio of bound protein in the polysaccharide-protein complexes was considered essential to the scavenging activity. In support of this, lentinan and schizophyllan, which contained only trace amounts of protein, exhibited no scavenging activities. In contrast, PSK (a protein-bound polysaccharide) and polysaccharide extracts form *Ganoderma* and *Grifola,* which had lower polysaccharide to protein ratios, showed the strongest scavenging activities [36].

Table 2 presents the antibacterial activity of lichen extracts from *X. somloensis* against clinical test bacteria. In all instances, more antibacterial activity was observed in the acetone extracts than methanol extracts. The acetone extracts exhibited antibacterial activity against *S. pyogenes, S. agalactiae* and *S. aureus* with inhibition zone of 30, 27 and 20 mm, respectively (Fig. 2). However, both extracts were found to be inactive against *E. coli* suggesting that these extracts did not inhibit Gram-negative bacteria. Lauterwein et al. [37] studied a lichen extract with potent activity only against Gram-positive bacteria while Hoskeri et al. [38] observed activity against both Gram-negative and Gram-positive bacteria. The reason for the different sensitivity between the Gram-positive and Gram-negative bacteria could be due to the different composition of the cell wall [39]. The cell wall of the Gram-positive bacteria consists of peptidoglucans (mureins) and teichoic acids, whereas the cell wall of the Gram-negative bacteria consists of lipopolysaccharides and lipopolyproteins [40].

Fig. 2. Antimicrobial activity of acetone extract from lichen *X. somloensis* against a clinical culture of *S. aureus*

The MIC value of both *X. somloensis* extracts with 3 clinical strains - *S. aureus, S. pyogens* and *S. agalactiae* - were found to be 0.8, 0.7, and 0.9 mg/ml, respectively (Table 2). In comparison, Behera et al. [24] calculated the MIC value of methanol, acetone and light petroleum extracts from lichen *Usnea ghatensis* against *S. aureus* and three *Bacillus* sp. in the range from 5 to 10 µg/ml. The MIC of acetone and methanol extracts from lichen *Lecanora frustulosa* were within the range of 0.78 to 3.12 mg/ml for *Bacillus mycoides, B. subtlis, Enterobacter cloacae, E. coli, Klebseilla pnemoniae,* and *S. aureus* [41]. Our study is the first report on antibacterial activity of extracts from the lichen *X. somloensis.*

CONCLUSIONS

In this work, the antioxidant and antibacterial activity of methanol and acetone extracts from the lichen *Xanthoparmelia somloensis,* native to the Black Hills in South Dakota, USA, were examined. It was shown that both the antioxidant and antibacterial activities correlated well with the protein to polysaccharide ratio rather than the polyphenol content of the extracts. It was demonstrated for first time that extracts from *X. somloensis* exhibited a strong antimicrobial activity against clinical test bacteria which could be of significance in human therapy, and treatment of animal and plant diseases. Work is underway to assess the antitumor and antiviral activities of the *X. somloensis* extracts.

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